Electronic Supporting Information

Controlled Intracellular Generation of Reactive Oxygen Species in Human Mesenchymal Stem Cells Using Porphyrin Conjugated Nanoparticles

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Supporting Experimental

Supporting Materials

N,N'-methylenebisacrylamide. Acrylamide, ammonium persulphate (APS). N.N.N'.N'tetramethylethylenediamine (TEMED), Brij L4®, dioctyl sulfosuccinate sodium Salt (98%)(AOT), 3acrylamidopropyl tetramethyl ammonium chloride (ACTA), copper (I) bromide (99% trace metal basis), fluorescein isothiocyanate dextran 10,000 MW (FITC-D), ammonium hexafluorophosphate tetrabutylammonium chloride, M199 medium, dimethylsulfoxide (99.5 %) and foetal bovine serum (FBS) trypsin, ethylenediaminetetraacetic acid (EDTA) 4-trifluoromethyl-7-hydroxycoumarin, XPhos, palladium acetate, N-methylmorpholine, magnesium sulphate bis(pinacolato)diboron, and Celite® were purchased from Sigma-Aldrich. Hexane, absolute ethanol, ethyl acetate, chloroform, methanol, acetonitrile ethyl acetate petroleum-ether 40-60 (Pet - Ether) and tetrahydrofuran (THF) were purchased from Fischer Scientific. Hanks balanced salt solution (HBSS) was obtained from Gibco. MitoTracker® red and green and LysoTracker® blue were obtained from Life Technologies. Fluorochrome-conjugated antibodies CD29 (FITC), CD105 (phycoerythrin (PE)), CD34 (phycoerythrin-237 (PC5)) and CD45 (phycoerythrin-Texas Red-X, ECD) were obtained from Beckman Coulter. Argon, nitrogen and carbon dioxide were purchased from BOC (UK). HPLC grade water obtained from a Maxima USF ELGA system (UK). Deionised water (18.2 M Ω) was generated by Elga Purelab Ultra (ULXXXGEM2).

Supporting Methods

Synthesis of positively charged alkyne functionalized nanoparticles

Surfactants Brij L4[®] (3.080 g) and AOT (1.590 g, 3.577 mmol) were dissolved in deoxygenated hexane (42 mL). Separately, a monomer solution* was prepared by mixing acrylamide (490 mg, 6.894 mmol), *N*, *N*'-methylenebis(acrylamide) (160 mg, 1.038 mol), *N*-propargylacrylamide¹ (25 mg, 0.110 mmol) and ACTA (20 mg, 0.073 mmol) made up to 2 mL with deionized water. An inverse microemulsion was established through addition of the monomer solution (aqueous phase) to the stirring surfactant-hexane solution (oil phase). APS (30 μ L, 10 % w/v) and TEMED (15 μ L, 0.100 mmol) were added to the stirring mixture to initiate polymerisation. After 2 hours of stirring hexane was removed by rotary evaporation (Buchi Rotavapor R-200). Nanoparticles were precipitated and washed with absolute ethanol (30 mL) using centrifugation (6 times, 6000 rpm, 6 minutes) with a Hermle centrifuge (Z300). After the final wash the pellet was resuspended in a small volume of absolute ethanol (10 mL) and vacuum filtered until dry (0.02 μ m pore filter, Sartorius Stedim Biotech). Dry nanomaterial (559 mg, 79.9%) was stored in a light protected container at – 18 °C.

* FITC-D (20 μ l, 5 mg/mL) was added to the monomer solution for cell uptake studies, to track the subcellular location of porphyrin functionalized nanoparticles.

Synthesis of zinc (II) and copper (II) centred porphyrin

Zn (*II*) *functionalized porphyrins:* Zn (II) functionalized porphyrins (5-4-[2-(azidoethoxy)ethyl]phenyl-10,15,20-tris-[(4-methylpyridynium)yl]porphyrinato Zn (II)trichloride) were synthesised as described by Giuntini *et al.*¹

Cu (*II*) *functionalized porphyrins:* 5-(4-azidophenyl)-10,15,20-[(4-methylpyridinium)yl]porphyrin trichloride (100 mg, 0.120 mmol) and Cu (II) acetate (50 mg, 0.250 mmol) were dissolved in deionised water (10 mL). The reaction mixture was stirred at room temperature for 15 minutes followed by addition of ammonium hexafluorophosphate (10%). The newly synthesised porphyrin precipitates out of solution and was recovered using centrifugation. The recovered pellet was dissolved in acetone and treated with tetrabutylammonium chloride. Cu (II) functionalized porphyrins (5-4-[2 azidoethoxy)ethyl] phenyl-10,15,20-tris-[(4-methylpyridynium)yl]porphyrinato Cu (II) trichloride) was isolated by filtration and purified by crystallisation (methanol/diethyl ether) to produce a red solid(103 mg, 96.0 %). Melting point > 300 C°; UV (H₂O): nm (int %): 425 (100), 549 (6.9), 590 (1.4); log ϵ_{425} : 5.16; t_R (min): 10.45.

Conjugating porphyrins to alkyne-functionalized nanoparticles via click chemistry

Porphyrins were covalently linked to nanoparticles *via* a Cu (I)-catalysed alkyne-azide cycloaddition reaction. The percentage of nanoparticle functionalization was determined by titrating unfunctionalzed nanoparticles with porphyrins. Nanoparticles saturated with porphyrin were considered to be 100 % functionalized. Nanoparticles functionalized with 5, 10 and 20 % Zn (II) or Cu (II) porphyrins were fabricated through addition of Zn (II) or Cu (II) porphyrins (5 % - 0.02 µmol; 10 % - 0.04 µmol; 20 % - 0.08 µmol) to alkyne functionalized nanoparticles suspended in deionised water (50 mg/1mL). The reaction was catalysed through addition of CuBr (7.17 mg, 0.05 mmol, in DMSO (200 µL)). The reaction mixture was made up to 2.5 mL with deionised water and allowed to stir in a light protected container overnight. Porphyrin nanoparticle conjugates were purified using PD10 desalting columns (GE Healthcare) and washed with absolute ethanol (30 mL) using centrifugation (2 times, 6000 rpm, 6 minutes). The porphyrin functionalized nanoparticle conjugates were dried in vacuum (~40 mg, 80 %).

Nanoparticle characterisation

Size: Nanoparticle size was determined by dynamic light scattering (DLS) (Malvern Nano-ZS, ZEN 3600, He-Ne 633 nm Laser, 5mW). Size measurements were obtained using three independent nanoparticle suspensions (2.5 mg/mL, HEPES pH 7.4).

Charge: The zeta potential of nanoparticle suspensions (2.5 mg/mL, HEPES pH 7.4) was determined using a zetasizer (Malvern Nano-ZS, ZEN 3600) and disposable zeta cells (DTS1060C). Zeta potentials were determined using Smoluchowski approximation consisting of three independent measurements (100 runs, 10 second delay between runs).

Fluorescence: Fluorescence spectra were recorded for nanoparticle suspensions (2.5 mg/mL) using a Varian Cary Eclipse fluorescence spectrophotometer.

Chemical synthesis of the H₂O₂-responsive reagent 7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-4-(trifluoromethyl)-2H-chromen-2-one (BPTFMC)

Synthesis of precursor 2-oxo-4-(trifluoromethyl)-2H-chromen-7-yl trifluoromethanesulfonate: 4-Trifluoromethyl-7-hydroxycoumarin (0.500)2.17 mmol), *N*-phenyl*bis*(trifluoro g, methanesulfonamide) (0.815 g, 2.28 mmol) and sodium carbonate (1.20 g, 10.86 mmol) were added to a dry two-neck round bottom flask. Then, dry DMF (20 mL) was added via syringe and the reaction stirred under a nitrogen atmosphere at room temperature for 15 min. The reaction was monitored by TLC (hexane:CHCl₃, 90:10, $R_f = 0.49$). Following completion of reaction, the mixture was filtered, the solvent removed under reduced pressure and the residual material was dissolved in CH₂Cl₂ (50 mL). and extracted with water (2 x 50 mL). The organic extract was dried (MgSO₄) and evaporated to dryness in vacuo to afford the 4-trifluoromethylcoumarin triflate as white solid (572 mg, 73%). M.p. 74-76 °C. v_{max} (KBr): 3492 (br), 3107 (s), 1751 (s), 1608 (s) cm⁻¹. ¹H-NMR (CDCl₃, 400 MHz): δ 7.84 (dq, $J_1 = 9$ Hz, $J_2 = 1.7$ Hz, 1H, C5-H), 7.37 (d, J = 2.47 Hz, 1H, C8-H) 7.31 (dd, $J_1 = 9$ Hz, $J_2 = 2.4$ Hz, 1H, C6-H), 6.87 (d, J = 0.4 Hz, 1H, C3-H). ¹³C-NMR (CDCl₃, 100 MHz): δ 157.57, 155.09, 151.65, 140.60 (q, ${}^{2}J_{C,F}$ = 33 Hz), 127.43 (q, ${}^{4}J_{C,F}$ = 2.4 Hz), 122.62 (q, ${}^{1}J_{C,F}$ = 275 Hz), 119.89, 118.49, 117.19 (q, ${}^{3}J_{C,F}$ = 5.4 Hz), 113.70, 111.35.

Synthesis of BPTFMC: 4-Trifluoromethylcoumarin triflate (300 mg, 0.828 mmol), XPhos (39.4 mg, 0.0828 mmol, 0.1 eq) and Pd(OAc)₂ (9.28 mg, 0.041 mmol, 0.05 eq) in a dry round bottom flask (25 ml) was added anhydrous THF (15 mL). N-Methylmorpholine (252 mg, 2.48 mmol, 3 eq) and bis(pinacolato)diboron (420 mg, 1.65 mmol, 2 eq) were then sequentially added to the stirred mixture. The reaction mixture was heated at 70 °C for 48 h, under nitrogen, in a light protected container and monitored by TLC (Hexane:EtOAc,80:20, $R_f = 0.6$). The resultant dark brown reaction mixture was cooled to room temperature, filtered through Celite® and the filtrate evaporated in vacuo to dryness. The residual material was dissolved in ethyl acetate (30 mL) and washed with brine (30 mL) and water (30 mL). The organic extract was dried over MgSO₄ and evaporated *in vacuo* to dryness to yield a pale yellow solid, which was further triturated with petroleum ether 40-60 to afford the title compound (50 mg, 18%). According to ¹H-NMR the purity of the product was ~95%; Analytical HPLC analysis (Phenomenex Monolithic- C_{18} (4.6 x 100 mm), flow rate 1.0 mL min⁻¹ and UV detection at 214 nm. Linear gradient: 10-90% solvent B over 18 minutes. Solvent A: 0.06% aqueous TFA; solvent B: 0.06% TFA in CH₃CN:H₂O, 90:10), $R_{\rm t} = 5.28$ min, 98% purity. M.p. 109-111 °C. $v_{\rm max}$ (KBr): 3426 (br), 2975 (s), 1753 (s), 1653 (s) cm⁻¹. ¹H-NMR ([D₆]-DMSO, 400 MHz): δ 7.73 (dq, $J_1 = 8$ Hz, $J_2 =$ 1.7 Hz, 1H, C5-H), 7.70 (dd, $J_1 = 8$ Hz, $J_2 = 0.8$ Hz, 1H, C6-H), 7.60 (apt, 1H, C8-H), 7.13 (d, J = 0.4Hz, 1H, C3-H), 1.32 (s, 12H). ¹³C-NMR ([D₆]-DMSO, 100 MHz): δ 158.56, 153.54, 139.33 (q, ${}^{2}J_{CF}$ = 32 Hz), 130.80, 124.67, 122.47, 121.88 (q, ${}^{1}J_{CF}$ = 275 Hz), 119.05 (q, ${}^{3}J_{CF}$ = 5.9 Hz), 115.73, 84.89, 25.01.

hMSCs collection, storage and preparation

Human fetal liver was collected into Roswell Park Memorial Institute (RPMI) medium containing penicillin/streptomycin, by the MRC-Wellcome Trust Human Developmental Biology Resource (Newcastle-upon-Tyne, UK); following appropriate maternal consents and ethical approval by the Newcastle and North Tyneside Research Ethics Committee, in accordance with Human Tissue Authority regulatory guidelines. Samples from a gestational age between 8 and 9 weeks, as determined using Carnegie staging were used in this study.² Upon receipt of the tissue (less than 24 hours) a single cell suspension was prepared through disruption of the fetal liver.³ Cell suspensions were transferred to RPMI, containing 10% DMSO and heat inactivated FBS (20 %) and stored in cryovials under liquid nitrogen vapour. Fetal liver-derived MSC cells were used for this study because they are well characterised and were readily available from ongoing studies.

For use, single cell suspensions were thawed at 37° C and washed in HBSS. Cells were resuspended in complete M199 medium, supplemented with heat inactivated FBS (20 %), L-glutamine (1% v/v) and penicillin/streptomycin (1% v/v). Fetal liver cells (5x10⁶) were plated in medium (7 mL) and placed in an incubator (37° C, 5% CO₂, 90% humidity). After 72 hours, non-adherent cells were discarded and adherent cells were washed twice in HBSS. At 80-90% confluence cells were harvested using trypsin/EDTA (Sigma) and subcultured in a ratio of 1:3. Adherent cells, regarded morphologically as mesenchymal stem cells (hMSC), were characterised after detachment, using flow cytometry and confirmed as hMSC according to literature.⁴ hMSCs from passages 3 to 8 were used for experimentation.

Delivery of porphyrin functionalized nanoparticles

hMSCs were cultured in 8 well plates (5x10⁴ cells/well, Lab-Tek Chamber Slide w / Cover Glass Slide) and allowed to adhere for 24 hours. Suspensions of (Zn (II) or Cu (II) 5, 10 or 20 %) porphyrin functionalized nanoparticles (2.5 mg/mL) were sterilised using a syringe filter (0.02 μ m pore). Cells were incubated with porphyrin functionalized nanoparticles for 10 hours (37°C, 5% CO₂, 90% humidity). Incubated hMSCs were washed twice with phenol-red and serum free medium (PR-S(-)M), to remove any non-internalized nanoparticles. Untreated hMSCs, utilised as negative controls, were subjected to identical experimental preparation.

Staining mitochondria, cumulative ROS production and determination of nanoparticles subcellular localisation

MitoTracker® red (5 μ L, 0.188 nM) was added to incubated cells to provide a fluorescent indicator to the intracellular location of mitochondria with active membrane potentials. After 20 minutes, non-internalized MitroTracker® red was washed away twice with PR-S(-)M. BPTFMC (5.2 nM), which will be transformed into fluorescent 7-hydroxy-4-trifluoromethyl-coumarin (HTFMC) in the presence of hydrogen peroxide, was added to incubated cells to provide an indirect indicator for cumulative ROS production. After 1 hour non-internalized BPTFMC was washed away twice with PR-S(-)M. To facilitate subcellular localisation of nanoparticles during uptake studies, the nanosensor matrix was doped with FITC-D (20 μ L, 5 mg/mL).

Fluorescence microscopy and controlled irradiation of hMSCs

Fluorescence microscopy: A DeltaVision Elite (Applied Precision) with Olympus IX71 stand inverted microscope coupled with an Olympus U-Plan S-Apo 60x 1.42 NA and 40x 0.95 NA (oil, Refractive index 1.520) objective was were used to image untreated hMSCs and hMSCs with internalised Zn (II) or Cu (II) porphyrin functionalized nanoparticles. A CoolSNAP HQ2 charged coupled device camera (6.45x6.45 μ m pixel cell, 1000 kHz), interfaced Resolve3D softWoRx Acquire(version 5.5.0) software was used to acquire images (1024 x 1024, pixel size 0.331 x 0.331 x 0.200 μ m). An InsightSSI solid state fluorescence light source was used to excite and collect fluorescence for fluorophores according to the parameters detailed in Table S1. The progress of cellular events was captured at 5 minute intervals, such that 5, 30, 60 and 100 minutes, corresponded to 1, 6, 12 and 20 irradiations, respectively. Captured images were analysed using FIJI open source software.

Controlled Irradiation: The InsightSSI solid state fluorescence light source was employed to irradiate hMSCs with controlled doses of excitation light during excitation of MitoTracker® red fluorescence using identical parameters (89 mW, 542/45, 632/, 2 seconds, 50 %). A power meter (LaserCheck Coherent), was used to determine the output from the Olympus U-Plan S-Apo 60x 1.42 NA objective (512 μ W, 8mm², 2 seconds).

Fluorophore	Microscopy Parameters				
	Power (mW)	λ_{ex} (nm)	λ _{em} (nm)	Exposure (seconds)	Transmission intensity (%)
HTFMC	55	390/18	435/48	0.8	100 %
FITC-D	54	475/28	523/36	0.3	75%
MitoTracker® Red	89	542/45	594/45	2.0	50%

Table S1. Description of microscopy parameters used to image hMSCs and internalised Zn (II) or Cu (II) porphyrin functionalized nanoparticles.

Flow cytometry and controlled irradiation using a custom built irradiator

hMSCs for flow cytometry: A Beckman Coulter flow cytometer (MoFlo XDP Cell Sorter) was utilised to study untreated and Zn (II) or Cu (II) porphyrin functionalized nanoparticle treated hMSC populations. hMSC preparation for flow cytometric analysis was analogous that for fluorescence microscopy using a minimum of 25000 cells/experiment. Healthy hMSC populations were selected through use of logical gates, and elimination of cell debris or dying cells using forward and side scatter.⁵ Flow cytometry analysis and plot generation was performed by Walter & Elisa analysis software (WEASEL: http://www.wehi.edu.au/other_domains/cytometry/WeaselDownload.php). Results are expressed as mean plus or minus standard deviation (SD). For statistical analysis one-way analysis of variance was performed, using Sigma-plot 11.0. P value <0.05 was considered statistically significant.

Staining: hMSCs were initially characterised to demonstrate their undifferentiated state using conjugated antibodies for cell surface markers: positive expression of CD29 (FITC) and CD105 (phycoerythrin (PE)), and absence of hematopoietic markers CD34 (phycoerythrin-237 (PC5)) and CD45 (phycoerythrin-Texas Red-X ECD).^{6, 7} A fluorescence-minus-one protocol was applied, as an isotype control, to eliminate non-specific antibody binding.⁸ Apoptosis was investigated using Alexa Flour® 488 Annexin V (5 μ L per 1x10⁶cells/mL), which binds to cell surface markers that are translocated during apoptosis, whereas, necrosis was determined using the DNA intercalator propidium iodide (1 μ L (100 μ g/mL) per 1x10⁶cells/mL) which stains deteriorating cells with permeable membranes. Annexin and propidium iodide were used to stain incubated cells for 20 minutes, and washed twice with PR-S(-)M to remove any non-internalised species. Antibodies, initially used to demonstrate undifferentiated state, were re-employed to identify induction of differentiation of hMSC secondary subcultures, subjected to 20 irradiations of excitation light.

Custom Built LED Irradiator: An irradiator was assembled through addition of neutral density filters (2x 0.3, Courtney Photonics) and an acrylic fluorescence filter (575 nm) to a light emitting diode (LED) light source (130 LEDs, 0.06W, LO201M (PRO ELEC), Figure S1. A power meter was used to measure the output of the light source ($500 \pm 5 \% \mu W$) and a spectrometer was used to determine the wavelength of excitation light (Ocean Optics USB2000+). hMSCs were dosed with 2 seconds of excitation light to mirror microscopy experiments.



Figure S1. (A) Custom built LED irradiator, coated with neutral density filters (x2) and acrylic fluorescence filter (575 nm). (B) Spectrum of output from custom built irradiator.

Supporting Results

Nanoparticle characterisation

Fluorescence: Zn (II) porphyrin functionalized nanoparticles phosphoresce, due to intersystem crossing of electrons to the triplet state. Figure S2A show emission spectra for nanoparticles functionalized with increasing quantities of Zn (II) porphyrin. Nanoparticles functionalized with higher percentages of Zn (I) porphyrin exhibit greater intensity, when excited at 575 nm. Zn (II) functionalized nanoparticle peak intensity is observed at 633 nm and increases linearly with Zn (II) porphyrin functionalization, Figure S2B.

Size: ACTA (2% w/w) and Zn (II) 5%, 10 % and 20 % porphyrin functionalized nanoparticles were found to have comparable hydrodynamic diameters centred at ~80 nm, Figure S3A.

Charge: The surface charge of a nanoparticle heavily influences its degree of uptake and subcellular localisation. Nanoparticles with positive surface charge often associate with the negatively charged cell membrane, which in turn facilitates their internalisation into sub-cellular compartments. The Page S5 of S10

porphyrins described in this article possess cationic functional groups. Nanoparticles were doped with ACTA, to maintain a minimum net positive surface charge of greater than +15 mV, to harmonise the degree of cellular uptake, Figure. S3B.

Cell viability and nanoparticle uptake: Nanoparticle associated toxicity and degree of uptake was assessed using flow cytometry. Initially forward and side scatter analyses were used to gate the viable hMSC population, Figure S4A. hMSCs were challenged with concentrations of 5% Zn (II) porphyrin conjugated nanoparticles, ranging from 2.5 - 50 mg/mL, Figure S4B-S4D. Reducing the nanoparticle concentration was found to increase the viability of hMSCs, as shown by reduction in side scatter, Figure S5A. A nanoparticle concentration of 50 mg/mL demonstrated a statistically significant decrease in cell viability (p<0.05, n =6). Increasing nanoparticle concentration did however increase the degree of nanoparticle uptake, which was assessed by quantifying the signal from Zn (II) porphyrins, Figure S5B. For this study we minimised cell toxicity, so that the effects of ROS production were attributed to the Zn (II) porphyrin functionalized nanoparticles.



Figure S2. (A) Phosphorescence intensity and (B) comparison of peak phosphorescence intensity for nanoparticles functionalized with 5, 10, 20, 50 and 100 % Zn (II) porphyrin.



Figure S3. Zn (II) 5%, 10% and 20 % functionalized nanoparticle (A) size and (B) zeta potential determined using dynamic light scattering and electrophoretic mobility, respectively.



Figure S4. Forward and side scatter bivariate plots for (A) untreated hMSCs and Zn (II) porphyrin functionalized nanoparticle treated hMSCs, at concentrations (B) 50 mg/mL, (C) 30 mg/mL and (D) 5 mg/mL, studied using flow cytometry.



Figure S5. (A) Flow cytometric analysis of cell viability of untreated and 5 % Zn (II) porphyrin conjugated nanoparticle treated hMSCs, at concentrations ranging from 50 - 2.5 mg/mL. Viable hMSC populations were identified by gating untreated hMSCs (*R1 Figure S4A*). * hMSCs treated with 50 mg/mL nanoparticles concentration were statistically different from untreated populations (one way ANOVA p<0.05, n=6). (B) Relative degree of 5% Zn (II) porphyrin functionalized nanoparticle uptake, for nanoparticle concentrations ranging between 50 - 5 mg/mL.



Figure S6. Larger inset images for of Figure 1A in main manuscript showing (i) bright-field image of hMSCs treated with (ii) LysoTracker[®] blue, (iii) MitoTracker[®] green and (iv) Zn (II) porphyrin conjugated nanoparticles (*red*).

Characterisation of BPTFMC

Sensitivity of BPTFMC – To determine the sensitive of BPTFMC to a variety of ROS, BPTFMC (5 μ M) was challenged with H₂O₂ (100 μ M), tert-butyl hydroperoxide (TBHP, 100 μ M), and hypochlorite (HOCl, 100 μ M). Hydroxyl radicals (OH•) and tert-butoxy radical (OtBu•) were generated by reaction of Fe⁺² (1 mM) with H₂O₂ (100 μ M) or TBHP (100 μ M), respectively, Figure S7. Sensitivity experiments were conducted in HEPES buffer (20 mM, pH 7.0, 37 °C)

Spectroscopic analysis of BPTFMC & HTFMC - Fluorescence spectra were recorded on a Varian Cary Eclipse fluorimeter, Figure S8A. Absorption spectra were recorded using a Varian Cary 50 spectrophotometer, Figure S8B. All measurements were made in HEPES buffer solutions (20 mM, pH 7) in quartz cuvettes. The molar absorption coefficient for BPTFMC and HTFMC was calculated to be 11870 and 13370 L.mol⁻¹ cm⁻¹, respectively, Figure S9.



Figure S7. Fluorescence response of BPTFMC (5 μ M, $\lambda_{ex} = 405$ nm) to hydrogen peroxide (H₂O₂), tert-butoxy radical (tBu) tert-butyl hydroperoxide (TBPH) hypochlorite (OCl-) and hydroxyl radical (OH•) at 100 μ M. The collected emission was integrated between 415 and 650nm.



Figure S8. Fluorescence excitation and emission spectra of BPTFMC and HTFMC



Figure S9. Beer Lambert plot for (A) BPTFMC and (B) HTFMC, where molar extinction coefficient are 11870 and 13370 L.mol⁻¹ cm⁻¹, respectively

Characterisation of hMSCs following light irradiation using flow cytometry.

Immediately after light exposure: Figure S10 shows untreated and Cu (II) or Zn (II) porphyrin functionalized nanoparticles show comparable scatter plots after 5 to 20 irradiations of excitation light. Untreated and Cu (II) porphyrin functionalized nanoparticle treated hMSCs were negative for hematopoietic markers CD34 and CD45 and positive for hMSC markers CD29 and CD105. Zn (II) porphyrin functionalized nanoparticles containing hMSC exhibited a normal phenotypic profile after 5 and 10 irradiations. However, a 3.8% and 5.7% relative decrease in expression of CD105 marker expression was observed after 15 and 20 irradiations, respectively.



Figure S10. Phenotypic characterisation of hMSC populations after irradiation with 5, 10 15 and 20 doses of excitation light.

After two subcultures: Figure S11 shows untreated and Cu (II) or Zn (II) porphyrin functionalized nanoparticles do not induce phenotypic changes in cells that have been passaged twice after 20 irradiations of excitation light.



Figure S11. Phenotypic characterisation of hMSC populations after irradiation with 5, 10, 15 and 20 doses of excitation light and two cellular passages.

Supporting References

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